QUANTITATIVE STUDIES UPON SOME BLOOD CONSTITUENTS OF HELIX POMATIA

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The living activities of *Helix pomatia* are in many ways dependent upon the weather conditions. During the cold months, from about October or November to March or April, the snails hibernate, closing their shells by secretion of the calcareous epiphragma, and receiving their energy from stored reserve substances. During the warm months the intensity of their activity depends largely upon the amount of rain falling in any particular year, the snails feeding and being active only in moist surroundings with the period of reproduction lasting usually from June to August. In a previous investigation (v. Brand, 1931) it was shown that the chemical composition of the body of *Helix* shows characteristic differences at various times during the annual cycle. It seemed of interest to study some of the blood constituents, in order to test whether similar regular changes occur here.

Material and Methods

The animals, *Helix pomatia,* were purchased from a dealer in Franconia. They were kept in an enclosure in a garden and fed regularly with lettuce and cabbage leaves. Broken pieces of *Helix* shells were provided to ensure a sufficient calcium supply. The snails lived under these conditions indefinitely with few animals dying. Every year, however, a new lot of freshly collected snails was added.

Almost every month of the years 1933 to 1936 the blood of two lots of snails (10 to 12 animals each) was collected by puncture of the lateral vein. The following determinations were performed: dry substance by drying a sample at 100° C., total inorganic substances by incineration of dry substance, P according to Embden (1921), Ca according to Holtz (1934). The Ca analyses were usually performed on an incinerated blood sample, although preliminary tests showed that a direct precipitation of the calcium with oxalate, as practised in analyses on human blood, is also possible. In the latter case the snail blood has to be diluted with 4 to 5 volumes of distilled water, because of the large amounts of calcium present. Cl was determined according to Austin and van Slyke

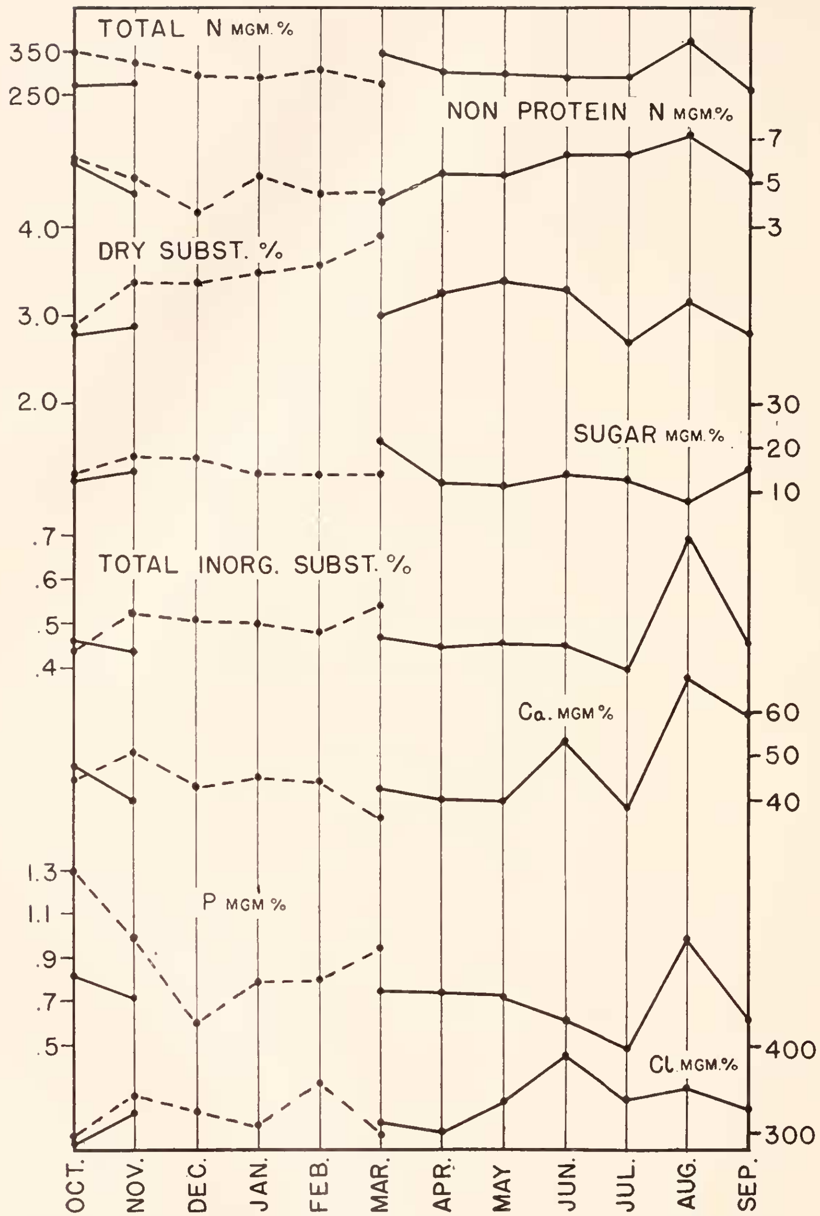

FIG. 1. Monthly averages of blood constituents of *Helix pomatia*. Solid line = Active life. Broken line = Hibernation.

(1920, 1921), sugar according to Hagedorn and Jensen (1923), total N and non-protein N according to a micro-Kjeldahl procedure. The values reproduced below for non-protein N are considerably lower than the figures of Wolf (1933), who reported values of roughly 20 mgm. per cent. This difference must be attributed to an incomplete precipitation of the proteins by Wolf. Dr. Wolf has since repeated his non-protein analyses in the laboratory of one of the authors (F. H.) and found the same low values that we give below. We used for the precipitation of the proteins a phosphomolybdenic reagent as prescribed by Bang (1927). Slightly higher values were found in preliminary experiments by using a mixture of tri-chlor-acetic acid with sodium-wolframate or, according to Folin, sodium-wolframate with ⅔ normal sulfuric acid.

TABLE I

Average values and maximal and minimal values of blood constituents of Helix pomatia.

	Average		Maximum		Minimum	
	Active life	Hibernation	Active life	Hibernation	Active life	Hibernation
Dry substance, *per cent*	3.1	3.4	3.9	4.0	2.0	2.4
Inorganic substances, *per cent*	.48	.50	.76	.63	.24	.40
Cl, *mgm. per cent*	331	322	450	395	220	265
P, *mgm. per cent*	.73	.91	1.80	1.50	.25	.36
Ca, *mgm. per cent*	48	44	133	82	28	7.9
Sugar, *mgm. per cent*	14	16	35	30	4	7
Total N, *mgm. per cent*	302	310	530	290	180	200
Non-protein N, *mgm. per cent*	5.6	4.9	14.6	8.4	4.4	3.0

Results

Fairly large variations of the monthly averages were apparent for all substances and they were in general decidedly more pronounced during the time of active life than during the period of hibernation (Fig. 1). Contrary to the findings reported previously for several constituents of the whole snail bodies, no significantly different trend of the blood constituent curves could be found during the different periods of the year. The extent of the variations present is demonstrated in Table I, where the maxima and minima both for the period of hibernation and of active life are shown. It is apparent that the minima are lower and the maxima are higher during active life. This is not surprising, since it is to be expected that with varying food and water intake during this time, the composition of the blood will be more variable than during the period of hibernation.

It should be noted that many of the substances tested (i.e. total inorganic, Ca, P, total N and non-protein N) showed a peak for their monthly averages in August (Fig. 1). With the exception of non-protein N the highest absolute values (Table I) occurred in this month. The explanation lies in the fact that August is the hottest and dryest month of the year. A certain concentration of the blood may be expected to result from these climatic conditions. In agreement with this

TABLE II

Mean values of blood constituents of Helix pomatia during the formation of the epiphragma. Extreme values are given in parentheses.

	Active life	During process of secretion of epiphragma	After formation of epiphragma
Dry substance, *per cent*	2.35 (2.04–2.68)	3.08 (2.60–3.92)	3.41 (2.78–3.73)
Inorganic substances, *per cent*	.42 (.34–.56)	.50 (.42–.58)	.45 (.40–.48)
Cl, *mgm. per cent*	290 (184–391)	302 (270–354)	311 (263–348)
P, *mgm. per cent*	.73 (.56–1.0)	.94 (.67–1.1)	1.24 (1.1–1.4)
Ca, *mgm. per cent*	38.4 (35.5–43.0)	38.5 (32.5–49.5)	37.9 (34.5–44.0)
Sugar, *mgm. per cent*	15 (7–21)	17 (9–22)	16 (11–21)
Total N, *mgm. per cent*	272 (187–306)	327 (290–358)	358 (318–382)
Non-protein N, *mgm. per cent*	5.5 (4.2–8.1)	5.8 (4.6–7.3)	5.9 (3.3–8.4)

assumption is the fact that the blood sugar was found to be very low in August—in a dry month the chances are small that a snail will feed much. Curiously enough, Cl and total dry substance were neither especially high nor low during August.

It should be kept in mind that many irregularities of the curves will be due to accidental causes, for example whether the animals had or had not received rain during a few days, or even hours before the analyses. Such conditions can change the picture from year to year. It might be mentioned as an example that in one year the monthly maximum for total N was found in August; in another year the animals analyzed in the same month gave values similar to the lowest of this whole series.

In order to test whether or not a significant difference between the blood composition during the periods of hibernation and of active life exist, the monthly averages of both periods have been averaged. In this way the accidental causes due to weather conditions are thought to be eliminated for the most part. It is quite apparent (Table I) that the values for both periods are identical. It seems likely that these values, which are based on a large number of determinations, represent the normal average level of these substances in the blood of *Helix*. It seems necessary to assume that in *Helix*, as in higher animals, a regulation of blood constituents is present. But the levels are not as fixed as in the latter, as demonstrated by the variations mentioned above.

It has been stated previously (v. Brand, 1931) that about half the inorganic substance occurring in a snail in autumn is used for the formation of the epiphragma. At the same time much water is lost from the body. It seemed of interest to study the blood composition during this period and in the spring when the epiphragma is shed. The animals intended for these determinations were kept under close observation in the fall of 1935 and 1936. Three groups of individuals were analyzed: (1) animals leading an active life, (2) animals in the process of secreting the epiphragma, and (3) animals having just completed its formation. The three lots were analyzed on the same days. They had therefore been subjected to the same climatic conditions before the analyses. In the spring of 1936 and 1937 the blood composition of snails at the end of the hibernating period and after having shed the epiphragma was investigated in a similar way.

It is apparent that during formation of the epiphragma (Table II) the dry substance content of the blood rises. This is due largely to an increase of the protein concentration, as evidenced by the considerable increase in total N. Non-protein N and sugar were practically unchanged.

Both the total inorganic substance and two of its components, Cl and Ca, showed almost no change. P showed a more distinct increase. The fact that the inorganic substance showed no greater changes during the formation of the essentially inorganic epiphragma, is rather remarkable. The epiphragma is secreted by glands located at the edge of the mantle. An accumulation of inorganic substances there is known to occur long before the epiphragma is actually secreted. However, Barfurth (1883) showed that the inorganic substances of the liver are greatly reduced after the formation of the epiphragma. Obviously, it is to be expected that they were transported to the mantle edge by the blood. That no rise in the blood occurs, may be explained on the assumption

TABLE III

Mean values of blood constituents of Helix pomatia at the end of the hibernation period. Extreme values are given in parentheses.

	Before shedding the epiphragma	After shedding the epiphragma
Dry substance, *per cent*	3.87 (3.32–4.20)	2.85 (2.56–3.05)
Total inorganic substances, *per cent*	.53 (.48–.60)	.42 (.34–.51)
Cl, *mgm. per cent*	302 (270–319)	274 (248–286)
P, *mgm. per cent*	1.37 (.95–2.00)	1.07 (.45–2.00)
Ca, *mgm. per cent*	31 (23–37)	30 (18–40)
Sugar, *mgm. per cent*	13 (7–16)	35 (29–47)
Total N, *mgm. per cent*	386 (319–588)	327 (300–372)
Non-protein N, *mgm. per cent*	6.3 (4.8–9.2)	4.7 (3.4–6.5)

that these substances are deposited in the glands at the same rate as that at which they are mobilized in other tissues.

As soon as the epiphragma was removed in the spring and the snail resumed an active life, the concentration of most substances tested

TABLE IV

Effect of moisture and food on blood constituents of Helix pomatia.

	Lot 1 (moist surroundings + food)	Lot 2 (moist surroundings, no food)	Lot 3 (dry surroundings, no food)
Dry substance, *per cent*	2.98	3.02	3.06
Inorganic substances, *per cent*	.60	.63	.67
Total N, *mgm. per cent*	295	270	258
Non-protein N, *mgm. per cent*	1.9	1.5	1.3
Dry substance of snail body minus shell, *per cent*	22.6	21.5	22.8

dropped more or less (Table III). This is doubtless due to the fact that a snail sheds its epihragma in general only if the moisture in the surroundings is high, i.e. after a rain. It then has an opportunity to take in water at once. A corresponding observation concerning the total body tissue has been published previously (v. Brand, 1931). It is curious to note, and as yet not explainable that the Ca content of the blood showed no change comparable to that of the other substances. The sugar content of the blood of free snails was found to have risen considerably. This may be interpreted as an alimentary hyperglycemia, since animals having shed the epiphragma begin immediately to feed.

Some experiments were performed in order to ascertain whether any blood constituents can be influenced readily under experimental conditions. They are of a rather preliminary nature, but are recorded here since we are at the present time not able to follow them up.

A short period of dryness, such as frequently occurs in nature, seems not to change the constitution of the blood to a marked degree. Fifty snails were divided into three lots as follows. Lot 1 was kept on moist

TABLE V

Blood sugar of Helix pomatia fed with cabbage soaked in 2 per cent dextrose solution.

Time	Blood Sugar *mgm. per cent*	Condition of Animals
0	8	Before feeding
1 hour	25	During "
2 hours	30	" "
3½ hours	49	" "
5½ hours	22	2 hrs. after feeding
7 hours	19	3½ " " "
9 hours	17	5½ " " "

filter paper with access to cabbage, lot 2 on moist filter paper but starving. Lot 3 was kept without food in an atmosphere dried by calcium chloride. The snails of lots 1 and 2 were active, those of lot 3 remained inside their shell most of the time, some closing the opening with a thin membrane. The blood composition after 5 days under these conditions is summarized in Table IV. There is no difference between the three lots.

The sugar content of the blood, on the other hand, can be influenced easily. A lot of snails was kept for some days without food or water. Then they were offered cabbage soaked in 2 per cent dextrose solution. They were allowed to feed for 3½ hours, following which they were again starved. At certain intervals during and after the feeding period the blood of some of the snails was collected and analyzed for sugar. A pronounced alimentary hyperglycemia was observed during the feeding period (Table V). The blood sugar sank slowly again as the snails

TABLE VI

Calcium content in normal and regenerating pieces of shell and in blood of Helix pomatia.

Lot	Days after removal of original piece	Ca Content		
		Removed piece *per cent total weight*	Regenerated piece *per cent total weight*	Blood *mgm. per cent*
1	0	—	—	38
2	7	44	10	94
3	14	34	33	42
4	24	36	32	34
5	34	35	?	32

were again starved, but it was still higher at the end of the experiment than at the beginning.

The last experiment deals with the question of whether the blood calcium rises when the snail is forced to regenerate a part of its shell. A fairly large piece of the shell was removed from some snails, and its Ca content was analyzed. After certain periods the Ca content of the piece regenerated and that of the blood was determined (Table VI). The Ca content in the regenerated piece reached the normal level after about two weeks. The value in lot 2 measured 7 days after the operation seems to indicate that the calcium content of the blood rises considerably in the early regeneration period. This is a contrast to the findings reported above concerning the formation of the equally calcareous epiphragma. Such a difference is not surprising. The formation of the epiphragma is a physiological process, whereas the regeneration of a large piece of shell is hardly a process to which the snail body is especially adapted. Small repairs, of course, occur frequently. Snails, for example, which had sealed together broken pieces of their shell, were fairly frequent in our material. We doubt, however, that under natural conditions snails would survive large defects to their shell.

Summary

1. Monthly analyses on different blood constituents of *Helix pomatia* showed that their concentration may vary considerably.

2. The mean values, gained from a large number of analyses, indicate, however, that there is no significant difference between the blood composition during the time of hibernation and that of active life. This is due to the fact that most, if not all, of the variability is due to climatic variations which balance out if observations are made over a long period

3. In snails forming the epiphragma the blood proteins and P were found in a certain concentration, but not the other inorganic substances.

4. The blood constituents become diluted by intake of water in snails shedding the epiphragma.

5. Short periods of dryness do not materially change the blood composition.

6. An alimentary hyperglycemia can be produced easily by feeding sugar to the snails.

7. In the early stages of shell regeneration the blood calcium is higher than normal.

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